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Differential Modulation of Cytokine Production by Macrolides: Interleukin-6 Production Is Increased by Spiramycin and Erythromycin

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Antibiotics do not act alone but act in conjunction with the host defense system. In particular, it has been shown that some antibiotics can modify cytokine production. We compared the in vitro effects of three macrolides (roxithromycin, spiramycin, and erythromycin) actively concentrated by leukocytes on interleukin-1α, (IL-1α), IL-1β, IL-6, and tumor necrosis factor alpha production by human monocytes stimulated with lipopolysaccharide. Our results show that the three macrolides tested have different effects on production of these cytokines. Spiramycin and, to a lesser extent, erythromycin increased total IL-6 production without affecting IL-1α, IL-1β, or tumor necrosis factor alpha production, whereas roxithromycin had no effect. To our knowledge, this is the first time that an antibiotic has been shown to increase IL-6 production.

Over the last 20 years, a growing interest in the study of interactions between antimicrobial agents and host defense systems has arisen. In effect, a wide range of antimicrobial agents administered in vivo or in vitro have been reported to modify host immune responses (14, 17, 31), including cytokine production (3, 4, 20, 29, 30).

Interleukin-1α (IL-1α), and IL-1β, IL-6, and tumor necrosis factor alpha (TNF-α) are the major cytokines produced by mononuclear phagocytes (6, 10, 23, 34). They mediate a wide range of biological activities that play a critical role in the induction of inflammatory and immune responses. Although these cytokines are biochemically and immunologically distinct proteins transcribed from different genes, they share a number of activities, such as the induction of fever and acute-phase protein synthesis by the liver (5, 12, 15, 26). They have also been found to exhibit synergy, and each can modulate the production of the others (1, 25, 33).

Macrolides are a class of antibiotics taken up and concentrated by leukocytes; as a result, they can reach intracellular concentrations far higher than those attained in the extracellular medium (8, 21, 28). This property may in turn alter the functions of phagocytes which are crucial for both antibacterial defense and inflammation, processes which are often interrelated.

The aim of this study was to investigate the effects of three macrolides (roxithromycin [ROX], spiramycin [SPI], and erythromycin [ERY]) on the capacity of peripheral blood monocytes isolated from healthy human volunteers to produce IL-1α, IL-1β, IL-6, and TNF-α in response to endotoxin stimulation.

MATERIALS AND METHODS

Drugs. ROX (glutamate; Roussel UCLAF), SPI (rovamycine adipate; Specia), and ERY (erythromycine IV lactobionate; Abbott) were kindly provided by the manufacturers. The drugs were dissolved in sterile water (1 mg/ml), stored at −20°C, and further diluted in RPMI medium to reach final concentrations of 1, 10, and 50 μg/ml; water was treated in parallel as a control.

Monocyte isolation. Human mononuclear leukocytes were isolated from heparinized blood of healthy adult donors by using Ficoll-Isopaque. The cells were then washed and resuspended in RPMI 1640 medium (J Bio) supplemented with 2 mM glutamine, 100 mM pyruvate, 1% nonessential amino acids (GIBCO), and 1% heat-inactivated fetal calf serum (Boehringer). Monocytes were counted by nonspecific esterase staining and adjusted to a concentration of 500,000 monocytes per ml of RPMI 1640. Monocytes were isolated by adherence (1 ml per well, 1.5 h, 37°C) to multiwell plastic plates (24 by 17 mm; Falcon). The nonadherent cells were then removed by three vigorous washings with Hanks' balanced salt solution. More than 95% of the adherent cells were monocytes, as determined by esterase staining.

Cytokine production. Following adherence, the cells were washed, and 1 ml of fresh serum-free medium was added to the monolayers with 10 μg of lipopolysaccharide (LPS) (*Escherichia coli* O.55:B_5; Difco) per ml and with or without various concentrations of the antibiotics (1, 10, and 50 μg/ml). Cultures were incubated for 18 h at 37°C in a humidified atmosphere of 95% air and 5% CO_2. To determine extracellular cytokine concentrations, cell-free supernatants were collected, centrifuged at 800 × *g* for 10 min, and stored at −70°C until assay. To determine cell-associated cytokine concentrations, the cells remaining in the wells were covered with 1 ml of fresh medium, lysed with 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propanesulfonate (CHAPS) (9 mM; Sigma) for 30 min, and stored at −70°C until assay. Addition of CHAPS to any of the recombinant cytokines used in this experiment did not modify the immunoassays.

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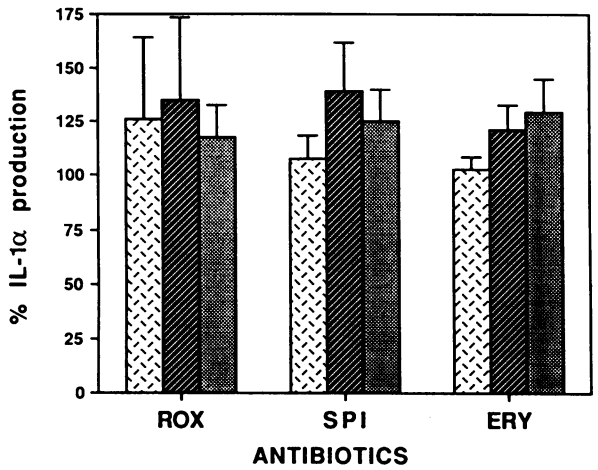

FIG. 1. Effects of ROX, SPI, and ERY on total IL-1α production. The effects of 1 (▨), 10 (▨), and 50 (▩) μg of ROX, SPI, and ERY per ml on total IL-1α production induced by 10 μg of LPS per ml for 18 h were evaluated by means of a specific ELISA. Results are expressed as (IL-1α production with antibiotic/IL-1α production without antibiotic) × 100 and are the means ± SEM of four different cell donors; experiments were performed in triplicate. LPS stimulation without antibiotics gave a total concentration of 0.247 ± 0.079 ng/5 × 10^5 cells with a ratio (extracellular versus cell-associated cytokine production) of 1/10.

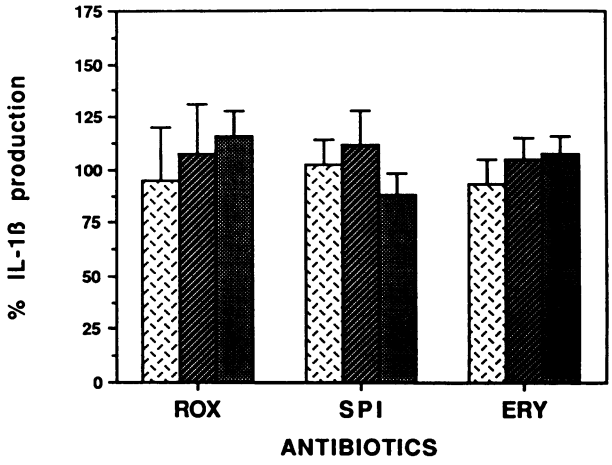

FIG. 2. Effects of ROX, SPI, and ERY on total IL-1β production. The effects of 1 (▨), 10 (▨), and 50 (▩) μg of ROX, SPI, and ERY per ml on total IL-1β production induced by 10 μg of LPS per ml for 18 h were evaluated by means of a specific ELISA. Results are expressed as (IL-1β production with antibiotic/IL-1β production without antibiotic) × 100 and are the means ± SEM of four different cell donors; experiments were performed in triplicate. LPS stimulation without antibiotics gave a total concentration of 16.29 ± 5.50 ng/5 × 10^5 cells with a ratio (extracellular versus cell-associated cytokine production) of 1/20.

Results were expressed as (total cytokine level with antibiotic/total cytokine level without antibiotic) × 100.

Cytokine immunoassays. Addition of ROX, SPI, or ERY to any of the recombinant cytokines used in this experiment did not modify the immunoassays.

IL-1α and IL-1β concentrations were determined by a specific enzyme-linked immunosorbent assay (ELISA) method as previously described (11). Briefly, Immulon 2-microtiter plates were coated with anti-human IL-1α or anti-human IL-1β. Anti-human IL-1α antibodies were raised in rabbits as previously described (11). Sheep anti-human IL-1β was a gift from S. Poole (National Institute of Biological Standards and Control, Potters Bar, England). Fifty-microliter aliquots of either appropriate standards (recombinant IL-1α or IL-1β) or unknown samples were dispensed into the coated wells, and 50 μl of horseradish peroxidase-conjugated anti-IL-1α or anti-IL-1β $F(ab')_2$ antibodies diluted in 0.2 M Tris buffer (pH 8.2, with 1% bovine serum albumin) was then added. The plates were incubated overnight at room temperature. Enzyme activity was determined with a color development solution consisting of 0.1 M phosphate-citrate buffer containing *o*-phenylene-diamine-2HCl (3 mg/ml). This method recognizes the mature as well as the precursor form of each IL-1 species with a detection limit of 25 pg/ml. There was no cross-reactivity between the detection of IL-1α and IL-1β (11). IL-6 concentrations were measured by an ELISA (IL-6; Medgenix) method. The detection limit is 15 pg/ml. TNF-α concentrations were measured by an immunoradiometric assay (Medgenix, Diagnostic Pasteur) method. The detection limit is 15 pg/ml.

Viability. Cell viability in the presence of antibiotics was checked after 18 h of incubation by the measurement of lactic dehydrogenase (LDH) activity in cell-free supernatants and cell lysates according to the method of Beutler (7). Results were expressed as (% LDH activity in culture supernatants with antibiotic/% LDH activity in culture supernatants without antibiotic) × 100.

Statistical analysis. Data were analyzed by using Student's paired *t* test. Values were expressed as means ± standard error of the mean (SEM) of four experiments.

RESULTS

Since cell-associated and extracellular cytokine production were influenced in a similar way, the results are given as total cytokine production. As shown in Fig. 1 through 3, ROX, SPI, and ERY at the concentrations tested (1, 10, and 50 μg/ml) did not significantly modify total IL-1α, IL-1β, and TNF-α production induced by LPS. Similarly, total IL-6 production was not modified by ROX at any of the concentrations tested (Fig. 4). In contrast, SPI significantly increased total IL-6 production in a concentration-dependent manner (from 8.29 ± 1.59 ng/5 × 10^5 cells, in absence of antibiotics, to 11.53 ± 2.00 and 13.55 ± 2.45 ng/5 × 10^5 cells at 10 and 50 μg/ml, respectively) (Fig. 4). ERY also increased total IL-6 production significantly but less potently (11.27 ± 2.08 ng/5 × 10^5 cells at 50 μg/ml) (Fig. 4). Cell viability was not altered by ROX, SPI, or ERY during the 18-h experimental period at any of the concentrations tested (Table 1).

DISCUSSION

Our results show that the macrolides tested had differential effects on cytokine production. SPI and, to a lesser extent, ERY increased total IL-6 production without affecting IL-1α, IL-1β, or TNF-α production, whereas ROX had no effect.

The action of antimicrobial agents on both humoral and cell-associated immunity is now recognized (2, 17). Tetracyclines, cephalosporins, macrolides, and quinolones have been shown to affect human monocyte responses (3, 4, 20, 29–31). Quinolones (ciprofloxacin, ofloxacin, and pefloxacin) have differential effects on LPS-induced cytokine produc-

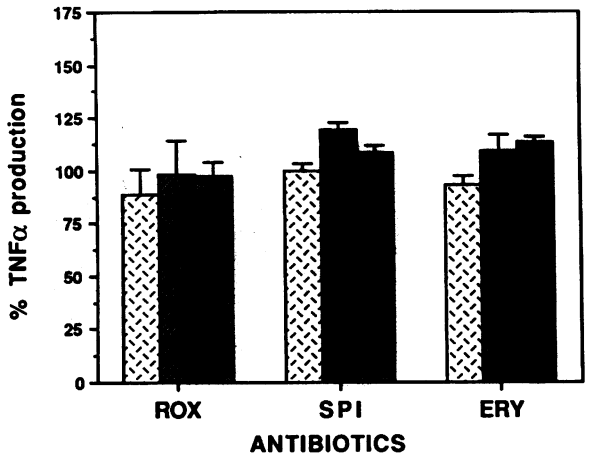

FIG. 3. Effects of ROX, SPI, and ERY on total TNF-α production. The effects of 1 (▨), 10 (■), and 50 (▩) μg of ROX, SPI, and ERY per ml on total TNF-α production induced by 10 μg of LPS per ml for 18 h were evaluated by means of a specific ELISA. Results are expressed as (TNF-α production with antibiotic/TNF-α production without antibiotic) × 100 and are the means ± SEM of four different cell donors; experiments were performed in triplicate. LPS stimulation without antibiotics gave a total concentration of 1.43 ± 0.27 ng/5 × 10^5 cells with a ratio (extracellular versus cell-associated cytokine production) of 10/1.

tion; at high concentrations, they inhibit IL-1β, IL-6, and TNF-α production without modifying total IL-1α production (3, 4). Cephalosporins also have differential effects on cytokine production; at high concentrations (200 μg/ml), they decrease IL-1α, IL-1β, and IL-6 production but not TNF-α production (3a).

The effects of SPI and ERY were observed at concentrations higher than 1 μg/ml. However, macrolides are a class

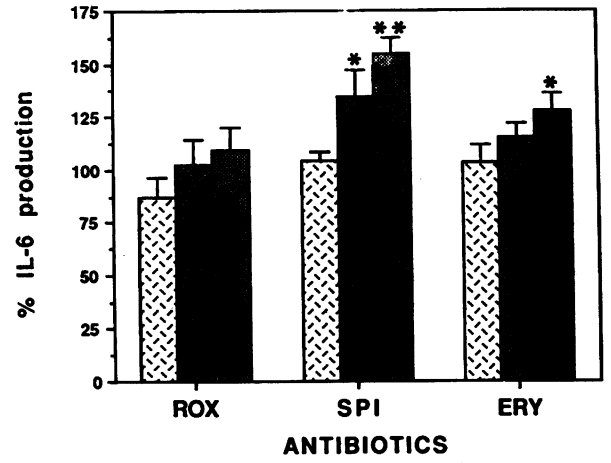

FIG. 4. Effects of ROX, SPI, and ERY on total IL-6 production. The effects of 1 (▨), 10 (■), and 50 (▩) μg of ROX, SPI, and ERY per ml on total IL-6 production induced by 10 μg of LPS per ml for 18 h were evaluated by means of a specific ELISA. Results are expressed as (IL-6 production with antibiotic/IL-6 production without antibiotic) × 100 and are the mean ± SEM of four different cell donors; experiments were performed in triplicate. LPS stimulation without antibiotics gave a total concentration of 8.29 ± 1.59 ng/5 × 10^5 cells with a ratio (extracellular versus cell-associated cytokine production) of 6/1. **, $P < 0.01$; *, $P < 0.05$.

TABLE 1. Effects of ROX, SPI, and ERY on cell viability[a]

Concn (μg/ml) of drug	% LDH activity[b]		
	ROX	SPI	ERY
1	95 ± 7	87 ± 8	82 ± 8
10	84 ± 6	82 ± 8	87 ± 12
50	105 ± 8	89 ± 9	74 ± 10

[a] Cell viability in the presence of antibiotics was checked after 18 h of incubation by the measurement of LDH activity.
[b] Results are expressed as (% LDH activity in culture supernatants with antibiotic/% LDH activity in culture supernatants without antibiotic) × 100 (mean ± SEM).

of antibiotics taken up and concentrated by leukocytes; as a result, they can reach intracellular concentrations far higher than those attained in the extracellular medium (8, 21, 28). Furthermore, their different pharmacokinetic properties, especially with respect to their biological half-lives and intracellular concentrations at a given time (9, 16, 27, 28), could explain their differential effects on IL-6 production within the same group. Indeed, differences in their interactions with phagocytes have already been shown (24, 32).

This is the first time, to our knowledge, that an antibiotic has been shown to increase IL-6 production. The involvement of IL-6 in human infections and cancers is clear (23, 34). Body fluids of patients with acute local bacterial or viral infections and the sera of patients with gram-negative or -positive bacteremia contain elevated levels of biologically active IL-6 (13, 18, 19, 35). The levels observed in the peripheral circulation can be on the order of 5 to 100 ng/ml. The ready detection of circulating IL-6 is consistent with the long-distance systemic role of IL-6 in mediating the host response to infection.

The relevance of our data to the clinical situation remains to be determined; the effects of SPI and ERY on IL-6 production were obtained at concentrations far higher than those attainable in serum, but these drugs have been reported to reach higher levels in some tissues, such as the tonsils and brain (22). Further studies with patients with infections will no doubt yield revealing data.

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